

STIC-ILL

RC 261. A1 C 2
me

From: Canella, Karen
Sent: Sunday, January 20, 2002 1:12 PM
To: STIC-ILL
Subject: ill order 09/802,457

Art Unit 1642 Location 8E12(mail)

Telephone Number 308-8362

Application Number 09/802,457

1. Clinica Chimica Acta, 1976 Jul 1, 70(1):103-112
2. Trans All-India Inst Ment Health, 1969, Vol 9, pp. 35-38.
3. Neurology, 1968 Apr, 18(4):397-402
4. Path Biol (Paris), 1963 Jun-Jul, Vol. 11, pp. 729-741
5. Clinical chemistry, 1989 Jun, 35(6): 972-974
6. Cancer, 2001 Aug 15, 92(4): 856-862
7. Revue Neurologique, 1992, 148(6-7): 417-422
8. Cancer Research:
1990 Oct 1, 50(19): 6364-6370
1987 Jul 15, 47(14):3766-3770
9. Cancer Bull, 1981, 33(6):250-254
10. Acta Neurochirurgica, 1971, 25(1):57-68
11. Neurology, 1968 Apr, 18(4):397-402
12. Int J of Cancer, 1996 Aug 22, 69(4):350-353
13. Clin Chem, 1997 Jan, 43(1):85-91
14. Calcif Tissue Int, 1997 Sep, 61(3):183-188
15. J Natl Cancer Inst, 1998 Jul 1, 90(13):1000-1008
16. Clin Cancer Research, 1999 Dec, 5(12): 3914-3919
17. Br J Haematol, 2000 Dec, 111(4):1118-1121
18. Thyroid, 1998 Aug, 8(8):637-641

Thanks!

Characterization of an Epithelial and a Tumor-associated Human Small Cell Lung Carcinoma Glycoprotein Antigen¹

Robert Waibel, Carl J. O'Hara, and Rolf A. Stahel²

Division of Oncology, Department of Medicine, University Hospital of Zürich, Switzerland [R. W., R. A. S.], and Department of Pathology, New England Deaconess Hospital, Boston, Massachusetts [C. J. O.]

ABSTRACT

The small cell carcinoma (SCC) antigens recognized by LAM2 and LAM8 antibody were characterized by comparison of their tissue expression and analysis of their biochemical composition. LAM2, but not LAM8 antigen could be demonstrated in lipid extracts of SCC cells. By immunohistochemical staining the SCC antigen LAM2 was shown to be an epithelial type membrane antigen. Immunoblotting experiments and competition solid phase radioimmunoassays showed LAM2 antigen to be a native conformation of a glycoprotein with major bands at *M*_r 100,000-120,000 and a minor band at *M*_r 210,000. L-Fucose was a dominant part of the epitope which appeared to be closely related to the carbohydrate epitope of the blood group antigen H(O). The tumor-associated membrane antigen LAM8 was shown to be a glycoprotein with major bands at *M*_r 90,000-135,000 and a minor band at *M*_r 200,000. Neuraminic acid was the predominant part of the carbohydrate epitope. LAM8 antibody recognized a structure in the saliva of Le^a positive probands, but untreated and neuraminidase-treated SCC extracts were unreactive with anti-Le^a antibody. Anti CA 19-9 (sialo-Le^a antigen) and LAM2 antibodies did not compete for LAM8 binding in direct radioimmunoassays. The sialo-GP₉₀₋₁₃₅ antigen recognized by LAM8 antibody therefore is likely to represent a novel tumor antigen.

INTRODUCTION

Several groups have reported monoclonal antibodies against membrane antigens of SCC³ cells. Many of these antigens have been identified as glycolipids, with a large fraction directed against the lacto-*N*-fucopentaose III (1-3). Characterization by immunoprecipitations suggested some other small cell carcinoma antigens to be proteins; these results, however, must be considered preliminary (4-6). Characterization by immunoblotting procedures have been reported on the SCC antigen MOC-1 which appears to be a protein of *M*_r 60,000 sensitive to formalin fixation (7).

We have previously reported on two antibodies reacting with small cell carcinoma cell lines and tissues. By indirect immunofluorescent staining, antibody LAM2 was found to react preferentially with small cell and squamous cell carcinoma, but also with normal bronchial epithelial tissues (8). In contrast, the antibody LAM8 was shown to react with small cell carcinoma tissue only (9). Both antigens were preserved after fixation in formalin. Preliminary immunoprecipitation experiments suggested LAM2 antibody might recognize a protein and immunoblotting experiments showed LAM8 antibody to react with a glycoprotein.

This report describes the characterization of the two SCC antigens recognized by the monoclonal antibodies LAM2 and

LAM8. The LAM2 antigen expression in formalin-fixed paraffin-embedded tissues was examined and compared with LAM8 expression. The glycoprotein nature of both antigens was elucidated, and the major carbohydrates of the respective epitopes were analyzed by immunoblotting and competition radioimmunoassays.

MATERIALS AND METHODS

Immunoperoxidase Staining of Tissues. Tissues fixed in alcohol-zinc-formol solution were cut in sections of 5 μ m and mounted on glued slides. The sections were deparaffinized and treated with methanol peroxide and 2% swine serum as blocking reagent. The sections were covered with antibodies at dilutions of up to 1:500 and incubated for 1 h at room temperature. After washes in 0.05 M Tris buffer, pH 7.6, peroxidase-conjugated swine anti-rabbit immunoglobulin diluted 1:30 and peroxidase-conjugated swine anti-rabbit immunoglobulin diluted 1:60 (DAKO Corp., Santa Barbara, CA) were added sequentially. The reaction was localized with 3,3'-diaminobenzidine tetramonohydrate (20 mg/10 ml in Tris buffer). Slides were counterstained with hematoxylin. Control studies included substitution with an irrelevant antibody (anti-leukocyte common antigen; DAKO).

Preparation of Monoclonal Antibodies. LAM2 and LAM8 are mouse IgM antibodies. Our procedure for antibody generation has been reported (8, 9). The antibodies were purified according to the method of Parham (10). Briefly, this was accomplished by applying the 30-55% ammonium sulfate fraction of the appropriate ascites fluid or the culture supernatant to a Sepharose 6B column (Pharmacia, Uppsala, Sweden) in PBS plus 0.5 M NaCl. The IgM positive peaks were pooled, precipitated by dialysis against 5 mM Tris, pH 7.0, and collected by centrifugation at 45,000 \times g for 1 h at 4°C.

Antibody Iodination. Purified LAM8 IgM was labeled with a solid phase protein iodination system (Protag-125; J. T. Baker Research, Phillipsburg, NJ) to a specific activity of 54 μ Ci/mg. Iodination was terminated by desalting the mixture on a small gel-filtration column (Bio-Gel P6-DG; Bio-Rad, Richmond, CA). Of the first peak eluted, 96% of the radioactivity was precipitable with trichloroacetic acid. The target uptake after labeling was determined and reached 71%.

Extraction, Enzymatic, and Periodate Treatment of Antigens. For biochemical characterization the small cell carcinoma cell line SW2 (Dr. S. D. Bernal, Dana-Farber Cancer Institute) was used. Cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum and 1 mM glutamine. For Western blot analysis, cells were washed twice in PBS and solubilized in 10 mM 3-(cholamidopropyl)dimethylammonio-1-propane sulfonate, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM leupeptine, and 50 μ g/ml pepstatin A (FLUKA, Buchs, Switzerland) in ice-cold PBS for 30 min. After centrifugation for 1 h at 130,000 \times g, the supernatant was collected for further analysis. For enzymatic digestion of the cell extract, proteins were precipitated in 80% cold acetone in order to eliminate protease inhibitors. After centrifugation, the pellets were dissolved in PBS and incubated at 37°C with the following enzymes: 0.2 unit neuraminidase; 1 unit proteinase K; 1 unit chymotrypsin (tosyl lysine chloromethyl ketone); 1 unit trypsin (*N*-tosyl-L-phenylalanylchloromethyl ketone) (all from Sigma); and 1 mg mixed glycosidases (mixture of exoglycosidases from *Charonia lampas* containing mannosidase, glucosidase, galactosidase, fucosidase, xylosidase, acetylglucosaminidase, and acetylgalactosaminidase) (Miles Scientific, Milano, Italy).

For periodate treatment of the antigens, detergent extracts were incubated with 10 mM periodate for 15 min at room temperature and the reaction was stopped with ethylene glycol.

Received 10/24/86; revised 3/16/87; accepted 4/15/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported by the Swiss Cancer League (FOR.302.85).

² To whom requests for reprints should be addressed, at Division of Oncology, University Hospital, CH-8091 Zürich, Switzerland.

³ The abbreviations used are: SCC, small cell carcinoma; PBS, phosphate-buffered saline (137 mM NaCl-2.7 mM KCl-8 mM Na₂HPO₄-1.5 mM KH₂PO₄); TBS, Tris-buffered saline (20 mM Tris-HCl-500 mM NaCl, pH 8.0); BSA, bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Immunological Detection of Antigens on Thin Layer Chromatograms. Glycolipids were isolated from SW2 cells as follows. Cell pellets (10^7 cells) were washed twice with PBS and lyophilized. The lipids were extracted using chloroform and methanol (2:1 by volume) with three times 2-min sonication. The extract was filtrated through a 0.22- μ m filter and evaporated on a Speed-Vac concentrator. Crude glycolipids were chromatographed on high performance thin layer chromatography silica plates (Machery-Nagel, Düren, West Germany) in chloroform:methanol:0.25% KCl, 5:4:1. After drying, the plates were either developed with ninhydrin spray reagent or immunostained as follows. The chromatograms were soaked for 2 h at room temperature in PBS and 5% nonfat dry milk to quench nonspecific binding. The plates were incubated overnight in supernatant with LAM2 or LAM8 monoclonal antibodies, washed twice for 30 min as before, and incubated with affinity-purified peroxidase-conjugated goat anti-mouse IgM(u) (Sigma), diluted 1:500 in PBS and 10% horse serum for 2 h. After washing as before peroxidase activity was demonstrated using 4-chloro-1-naphthol as substrate.

Immunological Detection of Antigens Transferred from SDS Gels to Nitrocellulose. Electrophoresis was accomplished on 10% SDS-polyacrylamide gels under reducing conditions using the buffer system of O'Farrell (11). Fifty μ g protein of the cell extracts were applied each. For LAM2 transfers, gels were first renatured 20 min each in 6 and 3 M urea and transfer buffer. Proteins were transferred electrophoretically onto 0.3 μ m nitrocellulose (Schleicher-Schull, Dassel, West Germany) according to the method of Towbin *et al.* (12) at 200 V, 1.5 A for 1 h, or 65 V for 15 h. The blotted nitrocellulose sheet was quenched by incubation (3×10 min) in TBS containing 5% nonfat dry milk (13). The sheet was then incubated for 16 h at room temperature with continuous rotation in LAM8, LAM2, NS-1 supernatant, or anti-Le^a or anti-Le^b antibody (Seraclone; Biotest; Dreieich, West Germany). After washing (3×10 min) in TBS with 5% milk and 0.05% Tween 20 the sheets were incubated with affinity-purified peroxidase-conjugated goat anti-mouse IgM(u) (Sigma), diluted 1:1000 in TBS and 5% milk for 1 h. After washing as before, peroxidase activity was demonstrated using 4-chloro-1-naphthol as substrate.

Radioimmunoassays. SCC cells were bound to 96-well plates as target for radioimmunoassays (14). The wells were coated with poly-L-lysine and 5×10^5 cells were fixed with glutaraldehyde to each well. The plates were stored at 4°C with 1% BSA and 0.2% sodium azide in PBS. For competitive inhibition of antibody binding to target cells, the optimal concentration of antibody was titrated using one-half of the maximal binding concentration. To test competition with monosaccharides, target plates were incubated with LAM2 antibody (20 μ l supernatant) or LAM8 antibody (25 μ l supernatant) in the presence of 100 μ l 0.1 M carbohydrate solution at pH 7.0 for 1 h at room temperature. To test binding competition with lectins, target plates were preincubated with 0.1 mg lectin/well for 1 h in PBS, washed twice in PBS and 1% BSA, and incubated with the respective amount of antibody in 100 μ l PBS and 1% BSA. To test competition with human saliva, plates were incubated in the presence of 20 μ l of saliva. After incubation, the plates were washed 4 times with PBS and 1% BSA, and ¹²⁵I-labeled goat anti-mouse IgM immunoglobulin (0.2 μ Ci/50 μ l) was added to each well. Each plate was again washed as before, and the individual wells were cut and counted in a gamma counter for 1 min. Typical counts for 100% binding were 60,000 cpm for LAM2 and 50,000 cpm for LAM8.

For competitive inhibition with radiolabeled LAM8 antibody, the plates were preincubated with 200 μ l of supernatant of LAM2 and NS19.9 (ATCC, Rockville, MD) cultures for 1 h, followed by 5 μ l (150,000 cpm) of labeled purified LAM8.

RESULTS

Expression of Antigens in Selected Tissues. The selective expression of LAM8 antigen in small cell carcinoma tissues has been reported previously. The expression of LAM2 antigen in tumor and normal tissues is summarized in Table 1 and the results compared to LAM8 expression. LAM2 and LAM8

Table 1 Expression of LAM2 antigen in formalin-fixed paraffin-embedded tissues and comparison with expression of LAM8 antigen

Tissue	No. imm.unoperox- idase positive/no. tested	
	LAM2	LAM8
Lung tumors		
Small cell carcinoma	8/9	9/12
Squamous cell carcinoma	2/2	0/4
Adenocarcinoma	3/4	0/4
Non-lung tumors		
Breast carcinoma	2/2	0/2
Adenocarcinoma of gastrointestinal tract	3/4	0/4
Ovarian carcinoma	5/5	0/4
Melanoma	3/3	0/2
Pheochromocytoma	0/1	0/1
Soft tissue sarcoma	0/3	0/3
Non-Hodgkin's lymphoma	0/5	0/2
Normal tissues		
Bronchus epithelium	4/4	0/4
Colon	0/3	0/3
Liver	0/2	0/2
Bile duct	2/2	0/2
Kidney	0/2	0/2
Pancreatic duct	3/3	0/2
Pancreatic islets	0/3	0/3
Breast duct	3/3	0/3
Ovary surface epithelium	3/3	0/3
Lymphatic tissue	0/4	0/4
Bone marrow	0/2*	0/2

* Megakaryocytes punctate positive.

antigens were preserved in formalin-fixed paraffin-embedded tissues. In contrast to LAM8 antigen, LAM2 was found to be expressed in lung carcinomas of every histology and adenocarcinomas of the breast, colon and ovary. Also, LAM2 was expressed in normal bronchus, breast ducts, and the surface lining of normal ovaries, but not in normal colon. LAM2 and LAM8 were not expressed in lymphomas, sarcomas, and normal mesenchymal tissues.

Western Blot Analysis of Antigens. Initial attempts to conventionally blot LAM2 antigen after direct transfer from SDS-PAGE to nitrocellulose failed. Subsequently, Western blots made on transfers of SCC membrane extracts renatured with 6 M urea showed specific bands with approximate molecular weights between 100,000 and 120,000 and at 210,000 (Fig. 1). Conventional Western blots of SCC membrane extracts with LAM8 antibody confirmed major bands between *M*_r 90,000 and 135,000 and revealed a second band around *M*_r 200,000 (Fig. 2). The reactivity of neither antigen differed in gels run under nonreducing conditions.

To further elucidate the nature of the antigens membrane extracts were treated enzymatically before separation on SDS-PAGE. Treatment with periodate, chymotrypsin, and trypsin completely abolished LAM2 reactivity. Treatment with neuraminidase resulted in the disappearance of about one-half of the bands in the molecular weight range of 100,000–120,000, whereas treatment with mixed glycosidases (mixture of exoglycosidases from *C. lampas* containing mannosidase, glucosidase, galactosidase, fucosidase, xylosidase, acetylglucosaminidase, and acetylgalactosaminidase) resulted in the visualization of several smaller molecular weight bands below 60,000 (Fig. 1).

Specific LAM8 reactivity was lost after treatment of cell extracts or nitrocellulose filters with periodate and proteinase K. Treatment with chymotrypsin reduced the size of the high molecular band and the major bands by about *M*_r 40,000. Binding remained unchanged after treatment with trypsin and

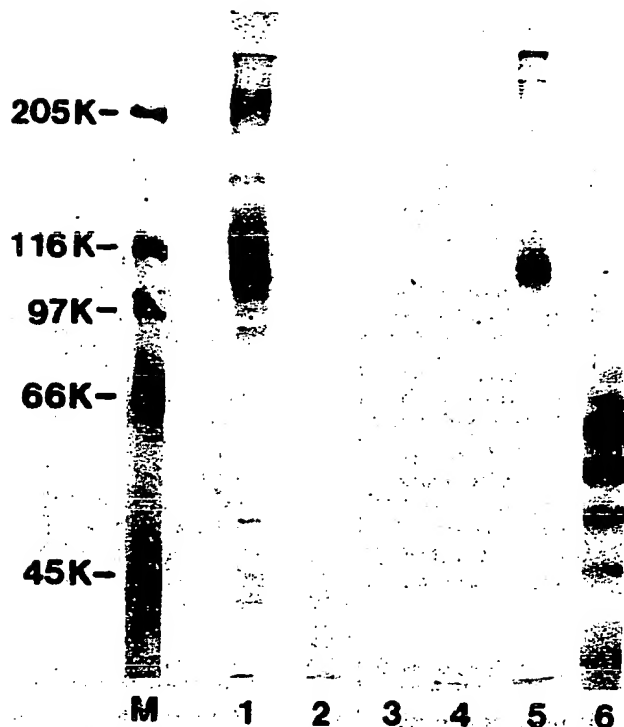


Fig. 1. Immunoblot of SCC extracts stained with LAM2 antibody. Molecular weight markers (M) are myosin (205,000), β -galactosidase (116,000), phosphor-lyase b (97,000), bovine albumin (66,000), and egg albumin (45,000). Lanes contain untreated extract (lane 1) and extract treated with periodate (lane 2), chymotrypsin (lane 3), trypsin (lane 4), neuraminidase (lane 5), and mixed glycosidases (lane 6). K, thousands.

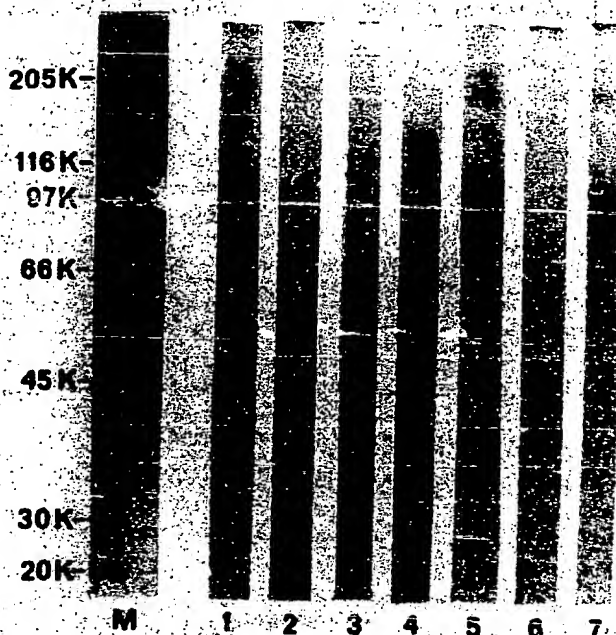


Fig. 2. Immunoblot of SCC extracts stained with LAM8 antibody. Lane 1, untreated extract, and extract treated with periodate (lane 2), proteinase (lane 3), chymotrypsin (lane 4), trypsin (lane 5), neuraminidase (lane 6), and mixed glycosidases (lane 7). M, molecular weight markers (see legend to Fig. 1); K, thousands.

mixed glycosidases. All reactivity was lost after digestion with neuraminidase (Fig. 2).

Relationship with Blood Group Antigens. A panel of RBC expressing defined blood group antigens was incubated with antibody. Agglutination and lysis (in the presence of human AB serum) was observed with LAM2 antibody in H(O)-positive

cells. LAM8 antibody had no effect on RBC with the blood group antigens A₁, A₂, B, O, Le^a, Le^b, M, and N. Antibody reactivity with saliva was examined by dot blot assays with SCC cell extracts serving as control. LAM2 recognized a component in saliva of group O secretors. LAM8 recognized a component in the saliva of Le^a positive probands.

SCC cell extracts were separated on SDS-PAGE, transblotted onto nitrocellulose, and in part treated with neuraminidase. A specific band was seen with LAM8 antibody but not with anti-Le^a antibody. Neuraminidase treatment of SCC extracts abolished LAM8 reactivity, but no reactivity with anti-Le^a antibody emerged (Fig. 3).

Presence of Antigens in Lipid Extracts. Chloroform-methanol extracts of SCC cells were separated by thin layer chromatography and the silica gels were incubated similarly to the nitrocellulose filters. With LAM2 antibody, antigenic reactivity could be demonstrated at the chromatography front. In contrast, LAM8 antigen was not demonstrable in lipid extracts (Fig. 4).

Epitope Characterization by Competition Radioimmunoassay. SCC cells were bound to microtiter plates and antibody binding was examined by indirect radioimmunoassays. To elucidate the structure of the epitopes, antibody binding was determined after enzymatic treatment of SCC cells or in the presence of potential inhibitors. Binding of both antibodies was not influenced after treatment of target cells with mixed glycosidases. LAM2 binding was not affected by treatment with neuraminidase, while LAM8 binding was reduced to less than 10% of control.

Antibody binding was also examined in the presence of acidic mucopolysaccharides and monosaccharides (Fig. 5). Binding of

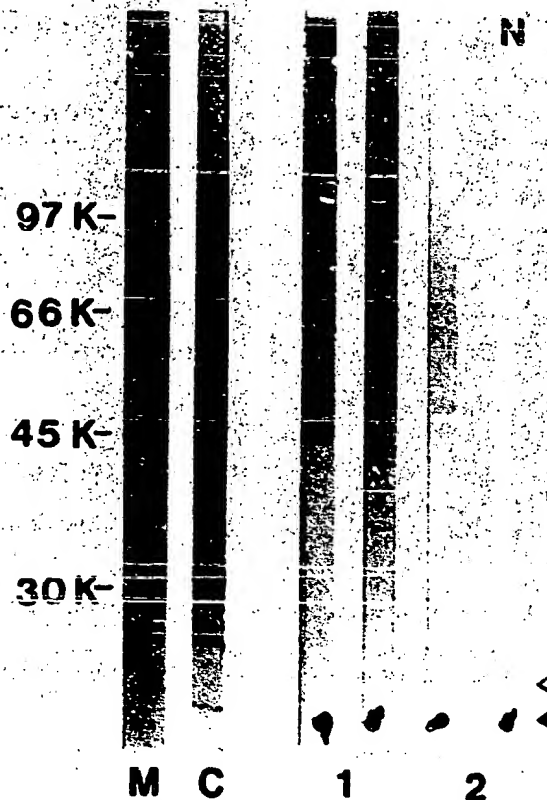


Fig. 3. Immunoblot of untreated and neuraminidase treated (N) SCC extracts. M, molecular weight markers (see legend to Fig. 1); C, control staining with amido black; lane 1, staining with LAM8 antibody; lane 2, staining with anti-Le^a antibody; \blacktriangle dot blot of Le^a saliva as positive control; \triangle dot blot of secretor saliva as negative control.



Fig. 4. Thin layer chromatograms of crude lipid extracts of SCC cells stained with ninhydrin reagent (N), LAM2 antibody (lane 1), LAM8 antibody (lane 2), and NS-1 supe. natant as control (lane 3).

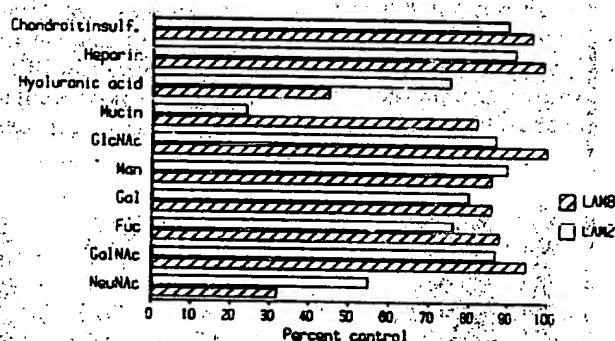


Fig. 5. Competition solid phase radioimmunoassay of SCC cells with LAM2 and LAM8 antibodies in the presence of mono- and polysaccharides. GlcNAc, N-acetylglucosamine; Man, mannose; Gal, galactose; Fuc, fucose; GalNAc, N-acetylgalactosamine; NeuNAc, neuraminic acid; Chondroitinsulf., chondroitin sulfate.

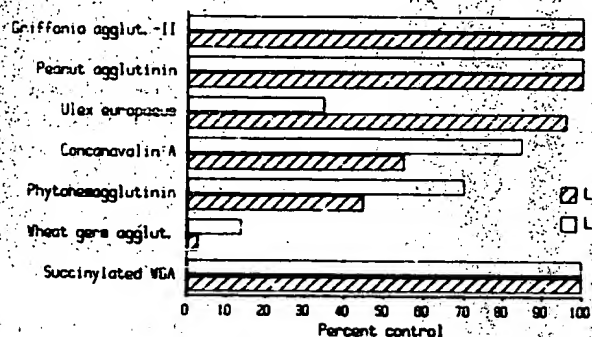


Fig. 6. Competition solid phase radioimmunoassays of SCC cells with LAM2 and LAM8 antibody after preincubation with lectins recognizing different carbohydrates: Griffonia agglut. (agglutinin) II, N-acetylglucosamine; Peanut agglutinin, galactose; Ulex europaeus, α -L-fucose; Concanavalin A, mannose; phytohemagglutinin, neuraminic acid and N-acetylglucosamine (1-3,4)- β -D-N-acetylglucosamine; Wheat germ agglut., neuraminic acid and N-acetylglucosamine dimers; Succinylated WGA, (wheat germ agglutinin), N-acetylglucosamine dimers.

LAM2 was inhibited in the presence of mucin and binding of LAM8 in the presence of hyaluronic acid. Heparin and chondroitin sulfate had no effect on antibody binding. Of various

monosaccharides tested, only neuraminic acid had a marked inhibitory effect. LAM2 binding in the presence of neuraminic acid was 55% and LAM8 binding 30% of control.

A series of lectins was used to specifically block potential antibody binding sites (Fig. 6). *Ulex europaeus* reduced LAM2 but not LAM8 binding. This lectin recognizes α -L-fucose and blood group H antigen. Wheat germ agglutinin recognizing neuraminic acid and N-acetyl- β -D-glucosaminyl residues reduced LAM2, and to a greater extent, LAM8 binding. This effect disappeared when succinylated wheat germ agglutinin (which has no affinity to neuraminic acid) was used.

Direct radioimmunoassays with radiolabeled LAM8 antibody were performed to examine competition of other antibodies for LAM8 binding. No inhibition of LAM8 binding was observed by the monoclonal antibodies LAM2, anti-19.9, and anti-Le^a.

DISCUSSION

In this paper we characterize two glycoprotein antigens of small cell carcinoma. The antigens differ in tissue expression and in molecular composition. Based on published results of immunohistochemical staining with antibodies directed against SCC membrane antigens, four major groups of antigens can be distinguished: (a) large number of antigens are also expressed in other epithelial structures, such as normal bronchial epithelium, nonsmall cell carcinoma of the lung, and nonpulmonary carcinomas such as breast or colon carcinoma. Immunohistochemical staining presented in this report showed LAM2 antigen to belong in this group of epithelial antigens, together with the antibodies SMI (15), E10/5, 2G3 (6), and PE-35 (6); (b) a second group of antigens appears to be associated with neuroendocrine differentiation. Examples include the antigen MOC-1 (7) and maybe B10/12 (6), SCLC5023 (16), and NE-35 (6). MOC-1 antigen was shown to be expressed in cells of neuroendocrine differentiation, in a small proportion of adenocarcinomas, but not in normal bronchial epithelium; (c) a third group of antigens was found to be commonly expressed in cells of macrophage differentiation or natural killer cells and SCC (17, 18); (d) a last group of antigens appears to be tumor associated, based on the absence of expression in normal bronchial epithelial tissues, other carcinomas, such as nonsmall cell lung, breast and colon carcinomas, tissues of neuroendocrine differentiation, and WBC. Based on results of tissue reactivity, LAM8 (9) and maybe SCLC5023 (16) would appear to recognize such a tumor-associated antigen.

Identification of surface proteins characteristic of small cell carcinoma by two-dimensional gel electrophoresis of radiolabeled membrane proteins has led to the hope of many investigators that their antibodies might be directed against such a protein (19). However, biochemical characterization of a large number of SCC antibodies by one group of investigators showed all their antigens to be glycolipids, many with the oligosaccharide lacto-N-fucopentaose as epitope. Our preliminary characterization of the SCC antigen LAM2 and LAM8 (8, 9) suggested the first to be a protein and the latter to be a glycoprotein antigen. The investigations reported in this communication were performed to better characterize these two antigens.

Work presented in this communication demonstrates LAM2 antigenic reactivity in crude lipid extracts and in a native confirmation glycoprotein. In immunoblots, LAM2 antigen could only be visualized after renaturation of the SCC extracts separated in SDS-PAGE. Specific bands had approximate molecular weights of 100,000–120,000 and 210,000. The antigen was sensitive to digestion with proteinases and mixed glycosi-

dases. L-Fucose was shown to be a component of the carbohydrate epitope. The epitope was shown to be related to the blood group antigen H(O) as demonstrated by the reactivity of LAM2 antibody with AB negative RBC and by inhibition of LAM2 binding of the lectin from *U. europaeus*.

Anti-tumor cell antibodies found to be reactive with the H-blood group determinant have been reported by other investigators. Examples in other respiratory tract tumors include antibodies against squamous cell carcinoma of the head and neck (20), lung adenocarcinoma (21), and the glycoprotein receptor for epidermal growth factor of the lung carcinoma cell line A431 (22). Earlier, a loss of AB isoantigens has been reported by immunohistochemical studies of nonsmall cell lung carcinoma with anti-AB sera (23). The uniform expression of the LAM2 antigen in SCC as well as in nonsmall cell carcinoma of the lung suggests that this phenomenon is common to lung tumors independent of their differentiation.

Immunoblotting experiments showed LAM8 antigen to be a glycoprotein with a major band at *M*_r 90,000–135,000 and a minor band at *M*_r 200,000. The antigen was sensitive to digestion with neuraminidase and chymotrypsin. Radioimmunoassays showed strong competition with neuraminic acid and the lectin wheat germ agglutinin. Thus demonstrating that neuraminic acid is a major part of the LAM8 epitope. Despite reactivity of LAM8 antibody with the saliva of Le^a positive probands, several lines of investigation suggested that LAM8 antigen was different from the Le^a blood group. No LAM8 reactivity was seen with Le^a positive RBC, and SCC cell extracts did not react with anti-Le^a antibody, even after treatment with neuraminidase.

LAM8 antigen shares sensitivity to neuraminidase with other tumor-associated antigens. The most extensively characterized of these antigens is CA 19-9, a sialylated Le^a antigen first identified by a mouse monoclonal antibody raised against colon carcinoma (24). Based on the lack of Le^a reactivity of neuraminidase-treated LAM8 antigen and the lack of competition between anti-CA 19-9 and LAM8 antibody, it can be concluded that the two antigens are not identical. However, despite its relatively low molecular weight, there are suggestions that LAM8 might belong to the group of mucin antigens because of its high carbohydrate content. Evidence for this is the relatively broad specific band after separation in SDS-PAGE and the inability for radioiodination of the antigen by both Bolton-Hunter or Protog methods (data not shown).

There is evidence that changes in sialylation of membrane glycoproteins might be related to the biological behavior of tumor cells. An increased membrane content of sialic acid was found to be associated with decreased transplantability of human and mouse melanomas (25), increased metastatic potential of murine tumor cells (26), and resistance to natural killer cell mediated cytotoxicity (27). Whether the membrane sialoglycoprotein identified by LAM8 antibody moderates similar biological reactions in small cell carcinoma remains to be determined.

Thus, our results show the SCC antibody LAM2 to define a native conformation epithelial type glycoprotein GP₁₀₀₋₁₂₀ with L-fucose as a dominant part in the carbohydrate epitope which appears to be closely related to the blood group H antigen. In contrast, the antibody LAM8 recognizes a tumor-associated sialoglycoprotein sGP₉₀₋₁₃₅ which is different from LAM2 and CA 19-9 (sialo-Le^a) antigen and is likely to represent a novel tumor antigen.

ACKNOWLEDGMENTS

We thank Dr. S. D. Bernal for providing the SW2 cell line and Dr. J. Gmür for performing hemagglutination experiments. We are grateful to Prof. G. Martz for his continued support.

REFERENCES

- Cutitta, F., Rosen, S., Gazdar, A. F., and Minna, J. D. Monoclonal antibodies that demonstrate specificity for several types of human lung cancer. *Proc. Natl. Acad. Sci. USA*, 78: 4551–4595, 1981.
- Brockhaus, M., Magnani, J. L., Herlyn, M., Blaszczyk, M., Steplewski, Z., Koprowski, H., and Ginsburg, V. Monoclonal antibodies directed against the sugar sequence of lacto-N-fucopentaose III are obtained from mice immunized with human tumors. *Arch. Biochem. Biophys.*, 217: 647–651, 1982.
- Rosen, S. T., Mushine, J. L., Cutitta, F., Fedorko, J., Carney, D. N., Gazdar, A. F., and Minna, J. D. Analysis of human small cell carcinoma lung differentiation antigens using a panel of rat monoclonal antibodies. *Cancer Res.*, 44: 2052–2061, 1984.
- Okabe, T., Kaizu, T., Fujisawa, M., Watanabe, J., Kojima, K., Yamashita, T., and Takuka, F. Monoclonal antibodies to surface antigens of small cell carcinoma of the lung. *Cancer Res.*, 45: 1930–1935, 1985.
- Reeve, J. G., Wulfrank, D. A., Stewart, J., Twentyman, P. R., Baillie-Johnson, H., and Bleehen, N. M. Monoclonal antibody defined human lung tumor cell surface antigens. *Int. J. Cancer*, 35: 769–775, 1985.
- Takahashi, T., Ueda, R., Song, X., Nishida, K., Shinzato, M., Namikawa, R., Ariyoshi, Y., Ota, K., Kato, K., Nagatsu, T., Imaizumi, I., Abe, T., and Takahashi, T. Two novel cell surface antigens on small cell lung carcinoma defined by mouse monoclonal antibodies NE-25 and PE-35. *Cancer Res.*, 46: 4770–4775, 1986.
- De Leij, L., Poppema, S., Nulend, J. K., ter Haar, A., Schwander, E., Ebbens, F., Postmus, P. E., and The, T. H. Neuroendocrine differentiation antigen on human lung carcinoma and Kulchitski cells. *Cancer Res.*, 45: 2192–2200, 1985.
- Stahel, R. A., Speak, J. A., and Bernal, S. D. Murine monoclonal antibody LAM2 defines cell membrane determinant with preferential expression on human lung small cell carcinoma and squamous cell carcinomas. *Int. J. Cancer*, 35: 11–17, 1985.
- Stahel, R. A., O'Hara, C. J., Mabry, M., Waibel, R., Sabbath, K., Speak, J. A., and Bernal, S. D. Cytotoxic monoclonal antibody LAM8 with specificity for human small cell carcinoma of the lung. *Cancer Res.*, 46: 2077–2084, 1986.
- Parham, P. Monoclonal antibodies against HLA products and their use in immunoaffinity purification. *Methods Enzymol.*, 92: 110–136, 1983.
- O'Farrell, P. H. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.*, 250: 4007–4021, 1974.
- Towbin, H., Staehelin, T., and Gordon, J. Electrophoretic transfer of proteins from polyacrylamide to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA*, 76: 4350–4354, 1979.
- JoLason, D. A., Grautsch, J. W., Sportsman, J. R., and Elder, J. H. Improved technique utilizing non-fat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. *Gen. Anal. Tech.*, 1: 328–334, 1984.
- Heusser, C. H., Stocker, J. W., and Gisler, R. H. Phase immunoassay for cell surface antigens. *Methods Enzymol.*, 73: 407–418, 1981.
- Bernal, S. D., and Speak, J. A. Membrane antigen in small cell carcinoma of the lung defined by monoclonal antibody SM1. *Cancer Res.*, 44: 265–270, 1984.
- Tong, A. W., Lee, J., and Stone, M. J. Characterization of two human small cell lung carcinoma reactive monoclonal antibodies generated by a novel immunization approach. *Cancer Res.*, 44: 4987–4992, 1984.
- Ruff, M. R., and Pert, C. B. Small cell carcinoma of the lung: macrophage-specific antigens suggest hemopoietic stem cell origin. *Science (Wash. DC)*, 225: 1034–1036, 1984.
- Bunn, P. A., Linnola, I., Minna, J. D., Carney, D., and Gazdar, A. Small cell lung cancer of the fetal bronchus, and other neuroendocrine cells express the Leu-7 antigenic determinant on natural killer cells. *Blood*, 65: 764–768, 1985.
- Baylin, S. D., Gazdar, A. D., Minna, J. D., Bernal, S. D., and Shaper, J. H. A unique cell-surface glycoprotein phenotype distinguishes human small-cell from non-small-cell lung cancer. *Proc. Natl. Acad. Sci. USA*, 79: 4650–4653, 1982.
- Kimmel, K. A., Carey, T. E., Judd, W. J., and McClatchy, K. D. Monoclonal antibody (G10) to a common antigen of human squamous cell carcinoma: binding of the antibody to the H type 2 blood group determinant. *J. Natl. Cancer Inst.*, 76: 9–19, 1986.
- Anger, B. R., Lloyd, K. O., Oettingen, H. F., and Old, L. J. Mouse monoclonal IgM antibody against human lung cancer line SK-LC-3 with specificity for H(O) blood group antigen. *Hybridoma*, 1: 139–146, 1982.
- Fredman, P., Richert, N. D., Magnani, J. L., Wiliingham, M. C., Pastan, I., and Ginsburg, V. A monoclonal antibody that precipitates the glycoprotein receptor for epidermal growth factor is directed against the human blood group H type 1 antigen. *J. Biol. Chem.*, 258: 11206–11210, 1983.
- Davidsohn, I., and Ni, L. Y. Loss of isoantigens A, B, and H in carcinomas of the lung. *Am. J. Pathol.*, 57: 307–334, 1969.
- Hansson, G. C., Karlson, K. A., Larson, G., McKibbin, J. M., Blaszczyk, M., Herlyn, M., Steplewski, Z., and Koprowski, H. Mouse monoclonal antibodies against human cancer cell lines with specificities for blood group and related antigens. *J. Biol. Chem.*, 258: 4091–4097, 1983.
- Berthier-Vergnes, O., Postoukalian, J., and Dore, J. F. Expression of cell surface glycoproteins in human melanoma cell lines with different tumorigenic properties. *Int. J. Cancer*, 36: 461–466, 1986.
- Yogeeswaran, G., and Salk, P. L. Metastatic potential is positively correlated with cell surface sialylation of cultured murine tumor cell lines. *Science (Wash. DC)*, 212: 1514–1516, 1981.
- Sherblom, A. P., and Moody, C. E. Cell surface sialomucin and resistance to natural cell-mediated cytotoxicity of rat mammary tumor ascites cells. *Cancer Res.*, 46: 4543–4546, 1986.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☒ **FADED TEXT OR DRAWING**
- ☒ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☒ **SKEWED/SLANTED IMAGES**
- ☒ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.